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Evaluating a Proposed Farm Best Management Practice: Nitrous Oxide Emissions, In-Bed Nitrate and Carbon Monitoring, and Hydraulic Retention Times of Denitrifying Woodchip Bioreactors in Monterey County, California

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EVALUATING A PROPOSED FARM BEST MANAGEMENT PRACTICE: NITROUS OXIDE EMISSIONS, IN-BED NITRATE AND CARBON MONITORING, AND HYDRAULIC RETENTION TIMES OF DENITRIFYING WOODCHIP BIOREACTORS IN MONTEREY COUNTY, CALIFORNIA

A Thesis

Presented to the

Faculty of the

Division of Science and Environmental Policy

California State University Monterey Bay

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Coastal and Watershed Science and Policy

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by

Christina M. David

Fall 2014

CALIFORNIA STATE UNIVERSITY MONTEREY BAY

The Undersigned Faculty Committee Approves the

Thesis of Christina M. David:

EVALUATING A PROPOSED FARM BEST MANAGEMENT PRACTICE: NITROUS OXIDE EMISSIONS, IN-BED NITRATE AND CARBON MONITORING, AND HYDRAULIC RETENTION TIMES OF DENITRIFYING WOODCHIP BIOREACTORS IN MONTEREY COUNTY, CALIFORNIA

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Dec. 18, 2014
Approval Date

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by

Christina M. David

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DEDICATION

I dedicate this thesis to those who helped me get there: to my family, who have been a pillar of support and strength to me always, and to others who have given me their time and motivation at various periods of my life.

I'd also like to recognize the Salinas Valley as a major inspiration to me, enabling me to continue to study aspects of the giant which is California agriculture.

On that note, if you have never been to see where your food is grown, I hope you consider making a trip to a farm or ranch. I will never forget the first time that I stepped onto a farm. I was 19 years old and on that sunny day, I found myself in a lima bean field in Chico alongside the professor who had hired me to work in his lab for the summer and the farm manager.

Having grown up in the suburbs, I didn't think of myself as a city girl. I'd been out in nature on plenty of occasions, but there was something poetic about the sound of wind rustling the wheat field adjacent to ours. Striding past the burms where the lima beans perched as tiny rivers of irrigation water flowed alongside them, I found myself in a world where nature had been seemingly harnessed.

Something about farms continues to hold glamor for me. However, that feeling of exhilaration I get from stepping onto a farm is also countered by a continuing education on farm worker rights, or the lack thereof. For example, I met workers who were in the field 6.5 days per week, over 10 hours per day, and this for ½ the wage that any high schooler is given at an entry-level position. Also, as an environmentalist as well as a budding agricultural enthusiast, I am rooting for more gentle treatment of our land, water, and animal resources. We cannot maintain good topsoil, and so we fertilize heavily despite impacts to the water supply and stream ecology. We waste water because it costs next to nothing, an inverted price compared to its unique value. We grow whatever crops we want where we want to, even if the land and the local water supply cannot support them.

I am hopeful that by reconnecting with our food, starting with where and how it is grown or raised, we will be more fulfilled on several fronts—cultural, ecological, and nutritional. That relationship—of knowing where and how your dinner was produced, and making decisions that affect both your personal health as well as the land and people that brought you what's on your fork—is a fulfilling one.

“It's a dangerous business, Frodo, going out your door. You step onto the road, and if you don't keep your feet, there's no knowing where you might be swept off to.” - J.R.R. Tolkien

ABSTRACT

Evaluating a proposed farm best management practice: Nitrous oxide emissions, in-bed nitrate and carbon monitoring, and hydraulic retention times of denitrifying woodchip bioreactors in Monterey County, California

by

Christina M. David

Master of Science in Coastal and Watershed Science and Policy

California State University Monterey Bay, 2014

Because surface waters in agricultural regions along the Central Coast of California have relatively high nitrate concentrations, there is a lot of interest to evaluate and implement various practices to improve water quality. This study assessed hydraulic retention times (HRTs), nitrate and carbon concentration gradients, and atmospheric fluxes and dissolved concentrations of nitrous oxide in three denitrification woodchip bioreactors on Salinas Valley farms that treat nitrate at 30 mg/L NO_3-N to 180 mg/L NO_3-N from field runoff. To evaluate HRT, we used sodium bromide tracer tests on two of the bioreactors, DBR1 and DBR 3, the former which had double the volume and flow rate. The mean HRT for DBR 1 was 41 hours and for DBR 3 was 35 hours, both of which were longer than expected. However, several system design parameters made the woodchip bioreactors non-ideal for salt tracer testing, as suggested by tracer stratification observed particularly during the DBR 3 test. Because of the potential for spatial heterogeneity of carbon and nitrate within the bioreactor beds that could indicate differential nitrate removal efficiency, we analyzed water samples from DBR 2 at various depths and distances along the bioreactor. Dissolved organic carbon availability decreased by 0.9 mg/L with a 0.3-m increase in depth, while nitrate increased by 4.3 mg/L with the same depth increase, both of which indicate that a greater extent of denitrification may be occurring near the surface of the bioreactor; however, this may or may not indicate greater nitrate removal efficiency, since if a slower flow path existed near the surface then nitrate removal would be increased but volume of water treated would be decreased. The volumetric nitrate removal rate of 6.7 g NO_3-N/m^3 bioreactor volume/day achieved by DBR 1 was high compared to other woodchip bioreactor studies, but because tile drain nitrate concentrations were also high, the two bioreactors at the tile drain sites only removed an average 12% to 19% of the incoming nitrate. Finally, few studies have evaluated nitrous oxide production from wood chip bioreactors, particularly atmospheric fluxes. To address this weakness, we installed static chambers in two of the bioreactors to measure nitrous oxide flux, and also took water samples to measure dissolved nitrous oxide. The mean atmospheric flux for DBR 2 was 2,700±1,300 µg N_2O-N/m^2/hour (mean±1 SD), while export of dissolved nitrous oxide was estimated as 424,000 µg N_2O-N/hour and 200,000 µg N_2O-N/hour for DBR 2 and DBR 3, respectively. These values were of similar magnitude to a New Zealand woodchip-and-sawdust bed study, much higher than two other woodchip bioreactor studies, and an order of magnitude higher than typical emissions from treatment wetlands. Therefore, although woodchip bioreactors have some potential as a nitrate-removing best management practice for the Central Coast, more work needs to be done to optimize the nitrate removal and explore if it is possible to limit their production of nitrous oxide.

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ACKNOWLEDGEMENTS

In rough chronological order, I'd like to thank Natalie Jacuzzi, a fellow graduate of the CWSP program, for giving me a collection of journal articles she had collected which included "Nitrate removal rates in a 15-year-old permeable reactive barrier treating septic system nitrate," which first spurred my interest in bioreactors. I'd like to thank my advisor Marc Los Huertos for the use of his lab, borrowing of his lab employees for advice and moral support, and of course for his guidance and multiple rounds of editing.

From the Monterey UC Cooperative Extension, I extend many thanks to Tim Hartz, Thomas Bottoms, Richard Smith, Patricia Love, and Thomas Lockhart for letting me assist in building one of the bioreactors, allowing me to conduct my study, fixing the bioreactors when they were being funky, and answering my smattering of questions over email.

I'd like to thank Daniel Muratore and Seneca Dykes for their field assistance during the nitrous oxide component of my study. Lab employees formerly mentioned include Stefanie Kortman and Erin Stanfield, who helped me analyze my data and taught me various things; and Pam Krone-Davis, another fellow CSWP graduate, who provided both field and analysis help and tips as well as positive vibes. I'd like to thank Gabriela Alberola for her help in writing a progress report on this project for the Leafy Greens industry and subsequently, a report. Thanks to Fred Watson for use of some R code.

Thanks also to Mitchell Vernon, David Hamblin, and Gwen Miller for helping with sampling and/or lab processing. Thanks to my cohort in this program for their energy and dedication: Scott Blanco, Brittany Bohlke, Alli Cramer, Cherie Crawford, Evan DeLay, Lisa Jensen, Shane Keefauver, Heather Kelley, Gwen Miller, Flower Moye, Polly Perkins, Shelley Petruccelli, Kirk Post, and John Silveus. Thanks also to our program's professors: Marc Los Huertos, Fred Watson, and Doug Smith, and also to John Skardon.

Lastly, a major thanks to the internship program by the USDA and CSU San Bernadino Water Resources Institute, which provided me with a stipend to do this project, as well putting on an annual conference and having gracious staff.

INTRODUCTION

Nitrogen is an essential element for all life; it is needed for plant growth and is also ubiquitous in human DNA and proteins. At elevated concentrations, however, reactive forms of it such as nitrate (NO_3^-) pose a threat to water quality, and nitrous oxide (N_2O) acts as a greenhouse gas in our atmosphere. Nitrogen enrichment in surface waters has been linked to increases in algal biomass which may negatively impact recreational use, drinking water quality, aesthetic value, allow for blooms of toxic algal species, elevate pH and deplete dissolved oxygen, and increase the probability of fish kills (Smith et al. 1999). To protect drinking water, the U.S. EPA set a Maximum Contaminant Level (MCL) of 10 mg NO_3-N/L for nitrate in 1992 (U.S. EPA 2014). In addition, the State Water Resources Control Board approved of the first Lower Salinas Total Maximum Daily Load plan for nitrogen and phosphorus, which addressed 35 regional waterbody/pollutant combinations in river and stream reaches that have been designated as "impaired" for these nutrients (SWRCB 2013). The plan requires that nitrate levels entering these impaired waterbodies should not exceed 8 mg/L NO_3-N/L or less, depending on the season and the specific stream reach.

Agricultural fertilizers have been identified as a major contributor of reactive nitrogen to waterbodies on a global, national, and regional level (Carpenter et al. 1998; US EPA 2014; Harter et al. 2012). The Salinas Valley is known for its agriculture, producing 70% of the nation's lettuce as well as diverse other specialty crops such as strawberries, broccoli, and artichokes. Of Monterey County's total 2.1 million acres, at least 290,000 is dedicated to row crops (Monterey Agricultural Commissioner 2013).

Best management practices such as constructed wetlands and planting of cover crops are recommended by the NRCS for nutrient mitigation. Woodchip bioreactors, also known as denitrification beds, are another such practice that could also serve to mitigate nitrate loads. They have been used to remove nitrate in several settings, including small septic systems and agricultural effluent, and have been studied in several countries as well as by the NRCS (Robertson et al. 2008; Schipper et al. 2010; Jaynes et al. 2008; Christianson et al. 2012). Woodchip bioreactors have some advantages over treatment wetlands in agricultural settings because they require less surface area, i.e. loss of productive land; Van Driel et al. (2006) reported achieving an order of magnitude greater

nitrate removal in units of area per day for their lateral flow bioreactor, as compared to reported values from numerous treatment wetland studies that were treating both surface and tile drain water sources. In addition, unlike wetlands, woodchip bioreactors do not have free-standing water nor require vegetation, which provides little habitat value for wildlife, a concern related to food safety management. Lastly, bioreactors are touted as being both low-maintenance and cost effective (Roberston et al. 2008; Harter et al. 2012).

The primary nitrate removal mechanism in woodchip bioreactors is biological denitrification. Under reducing conditions, heterotrophic bacteria use labile organic carbon to convert nitrate to dinitrogen and trace gases (Fig. 1) (Tiedje 1988; Seitzinger et al. 2006). Carbon availability is an important limiting factor in denitrification beds (Well et al. 2001). Slow-release carbon media such as woodchips are preferred due to their longevity, with bioreactor studies suggesting sustained pollutant removal for over a decade, although with this media carbon levels remain limiting (Cameron and Schipper 2010; Warneke et al. 2011). However, removal rates can be improved by faster-releasing amendments such as soybean oil, maize cobs, wheat straw, and alfalfa (Robertson et al. 2008; Greenan et al. 2006).

$$6NO_3^- + 5CH_3OH + 6H^+ \rightarrow 3N_2 + 5CO_2 + 13H_2O \qquad (a)$$

$$NO_3^- \xrightarrow{\text{nitrate reductase}} NO_2^- \xrightarrow{\text{nitrite reductase}} NO \xrightarrow{\text{nitric oxide reductase}} N_2O \xrightarrow{\text{nitrous oxide reductase}} N_2 \qquad (b)$$

Figure 1. ***(a)*** **Heterotrophic denitrification reaction, using methanol as an example carbon source; and** ***(b)*** **denitrication process showing intermediate nitrogen species (equation 2 from Christianson 2011).**

Another important factor in denitrification bed design is a sufficient hydraulic retention time, to optimize the contact time between denitrifying bacteria and dissolved constituents, i.e. organic matter and nitrate. Currently studies use different nitrate removal reporting metrics to compare inlet and outlet nitrate concentrations—as a percent, concentration difference, or mass removed per volume of woodchips (Christianson et al. 2012).

While numerous studies have shown that a greater percentage of nitrate that enters the bioreactor is removed by increasing retention time, and also that longer retention times may be necessary during the colder months since denitrification rates are reduced, achieving complete removal may or may not optimize the total load of nitrate removed by the bioreactors since a greater retention time means that less of the nitrate-laden water will be processed (Chun et al. 2009; Greenan et al. 2009; Christianson et al. 2012; Woli et al. 2010). For example, in a lab-scale study testing woodchips columns at flow rates of 2.9, 6.6, 8.7, and 13.6 cm d^{-1}, nitrate removal per gram of woodchip was shown to increase with flow rate (Greenan et al. 2009).

Injection and analysis of tracers in flow-through systems is a tool to determine retention time, and can reveal non-ideal conditions in wetland hydrology such as short-circuiting (by-pass flow) or dead zones. However, the tracer itself may be influenced by changes to water volume, such as by leaks or major evapotranspiration, and retardation due to dead zones or reversible/irreversible sorption (Kadlec and Wallace 2009).

Nitrous oxide, a greenhouse gas 300 times as potent as carbon dioxide by weight, has been shown to account for 0.003% to 3.3% of removed nitrate in woodchip bioreactors when measuring gas dissolved in the water matrix (EPA 2013; Greenan et al. 2009; Elgood et al. 2010; Moorman et al. 2010; Warneke et al. 2011a). Warneke et al. (2011a) measured high surface and dissolved nitrous oxide emissions, which combined accountied for 4.3% of removed nitrate. More studies of both dissolved nitrous oxide and atmospheric fluxes from woodchip bioreactors are needed on field-scale beds. Nitrous oxide is produced when the denitrification process is not complete and may also be the primary product of microbes missing a nitrous oxide reductase enzyme (Warneke et al. 2011b). Agriculture contributes an estimated 52% of California's nitrous oxide emissions, and 6% of California's total greenhouse gas emissions (ARB 2009). Factors influencing the rate of nitrous oxide emissions include degree of water saturation, temperature, pH, nitrogen and carbon concentrations as well as C:N ratio; complete saturation as well as lower temperature and pH are contended to drastically reduce emissions, while higher nitrate will increase emissions (Dobbie et al. 1999; Bouwman 1996; Brown et al. 2000; Maggiotto et al. 2000; Minamikawa et al. 2010; Firestone and Davidson 1989).

Lastly, high temperatures may stimulate nitrous oxide emissions, but also enhance denitrification rates overall. Soil microbes' denitrification rates are highest within some optimal temperature range, shown to be between 25 ˚C and 35 ˚C in several studies (Saad and Conrad 1994; Hallin et al. 2012). Natural temperatures found in bioreactors are less than this optimal range and therefore may benefit from, for example, solar-powered heating of beds (van Driel et al. 2006; Robertson et al. 2000). For every 10 ˚C increase in temperature, nitrate removal in bioreactors increases by a factor of 2, although there is some variability around this number (Hoover 2012; Cameron and Schipper 2010; Robertsion and Merkley 2009; Warneke et al. 2011a).

OBJECTIVES

Our objectives were to assess the internal dynamics of the bioreactors, including hydrology and other parameters related to nutrient removal, and to address the greenhouse gas potential of the beds. Specifically, our goals were to:

1) Evaluate hydraulic retention time (HRT) of bioreactors;
2) Evaluate temporal and spatial patterns of nitrate and carbon levels within the bioreactor; and
3) Quantify nitrous oxide emissions, both surface flux and also dissolved within the water matrix.

HYPOTHESES

1. We predicted higher nitrate concentrations at lower depths and conversely, lower dissolved organic carbon (DOC) at lower depths. These predictions were based on the results of preliminary samples analyzed at DBR 2. Nitrate concentrations were predicted to be roughly equivalent across the width of the bed, indicating evenness in the amount of denitrification and flow.
    a. $[\text{nitrate}]_{0.3\text{meter\_depth}} < [\text{nitrate}]_{0.6\text{meter\_depth}}$
    b. $[\text{DOC}]_{0.3\text{meter\_depth}} > [\text{DOC}]_{0.6\text{meter\_depth}}$
    c. $[\text{nitrate}]_{\text{left}} = [\text{nitrate}]_{\text{center}} = [\text{nitrate}]_{\text{right}}$
2. Atmospheric fluxes of nitrous oxide were not predicted to vary relative to chamber location within the bed.

a. $N_2O\ Flux_{near\text{-}inlet} = N_2O\ Flux_{mid\text{-}bed} = N_2O\ Flux_{near\text{-}outlet}$

3. Atmospheric fluxes of nitrous oxide were predicted to increase with temperature.
4. Atmospheric fluxes of nitrous oxide emissions were predicted to be higher on dates including methanol injection than on dates before and after methanol injection (20 ppm methanol). By injecting methanol, a supplemental carbon source, denitrification rates should increase, suggesting a potential for a corresponding increase in nitrous oxide emissions, notwithstanding the effect of a shifted C:N ratio.

a. $N_2O\ Flux_{with\_methanol} > N_2O\ Flux_{no\_methanol}$

DESCRIPTION OF SITES

This study assessed three bioreactors adjacent to farms in Monterey County, which reaches from the Monterey Bay coastline and extends through the Salinas Valley. Average summer temperatures, including nighttime low temperatures, range from 10 to 25 ˚C, while winter temperatures range from 4 to 18 °C.

Three woodchip bioreactors were installed and monitored by the Monterey County Cooperative Extension with the growers' and owners' cooperation, and were installed between 2011 and 2012 (Table 1). To maintain the growers' privacy, the UC Cooperative Extension naming scheme for the sites will be used here: DBR 1, DBR 2, and DBR 3 (denitrification bioreactor). The bioreactors all receive agricultural runoff

Table 1. Descriptive parameters of the bioreactors located in the Salinas Valley.

Site	Total bed volume (m^3)	Influent flow rate (liters/ min)*	Date installed	Runoff source	Seasonal temp. range (˚C) of bed	Influent nitrate concen-trations (mg/L)	Methanol injection dates (2013)
DBR 1	26	7.6	Apr. 2011	Tile drains	12-16	80-170	Apr. 25-May 29, Jul. 3-Aug. 1. Sept. 3-Sept. 20
DBR 2	13	3.8	Apr. 2011	Tile drains	13-17	60-120	Mar. 30 - Jul. 2, Aug. 1-Sept. 3
DBR 3	12.2	3.8	May 2012	Surface flows	16- 23	25-50	N/A

*** These rates were set by UC Cooperative Extension personnel, but did experience fluctuations due to equipment issues and water level fluctuations at where the influent was drawn by a pump (i.e. in the sump or the holding pond)**

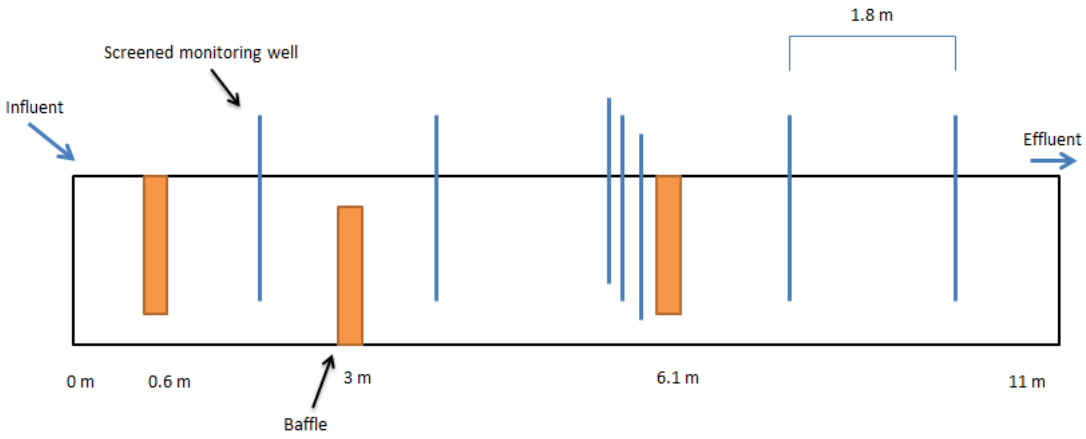
from one of two sources. DBR 1 and DBR 2 receive tile drain water, which flows beneath the field and into a sump, where it is then pumped to surface drainage ditches. DBR 3 receives runoff that flows directly off the surface of the fields and into drainage ditches, then into a series of two holding ponds, before entering the bioreactor. The runoff water at this site also carried much more suspended sediment than the other sites, so the water was treated with polyacrylamide to remove it immediately before entering the bioreactor.

The source of the runoff was from fields growing either leafy green vegetables or strawberries. At each site, untreated runoff has the potential to enter waters of the State. In some locations a local pumping station collects water from numerous drainage canals and pumps the untreated runoff into the Salinas River. The Reclamation Canal and the Old Salinas River channel are other possible recipients of runoff. These waterbodies all eventually flow into the Monterey Bay. This BMP was designed to reduce nitrate loading to waters of the State.

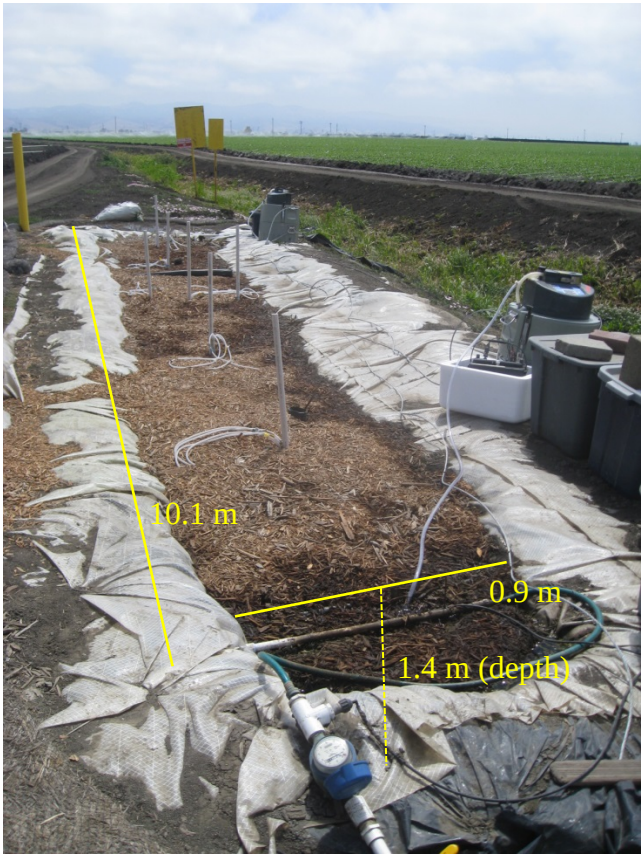
At DBR 2, methanol injection occurred for one month during the nitrous oxide sampling period. The purpose of the methanol injection was to determine the level of enhanced nitrate removal from supplementing the carbon source in the bioreactor. Other management procedures that occurred during the study period include woodchip replenishment, whereupon 150 kilograms of woodchips or more were added to each bioreactor annually. After replenishment, the bioreactor beds were packed tightly enough with woodchips that it was possible for a person to walk on them without any major compression or sinking. However, over the course of the year sometimes, as in the spring and summer of 2013, DBR 3 had a low woodchip density at the near-inlet section for several meters that could not support the weight of a person and likely had a negative effect on flow patterns and denitrification rates.

Upward- and downward-forcing baffles were installed in each bioreactor as design tools to increase HRT by creating a more sinuous flow path. DBR 1 contains two baffles; the first is upward-forcing and the second is downward-forcing (Fig. 2). DBR 2 and DBR 3 have identical baffle placement, with two downward-forcing baffles and one upward-forcing forcing one. Anaerobic conditions have been confirmed in these bioreactors with dissolved oxygen probes several times throughout the study. Monitoring

wells constructed from clear PVC tubing fitted with aeration stone sampling ports, attached to a PVC pipe frame, were screened at 0.3-meter depth increments.



(a)



(b)

Figure 2. (a) Diagram of DBR 2, with baffle and monitoring well placement; and (b) photograph of the DBR 2 bed with PVC monitoring wells visible. The inlet is in the foreground, and the outlet feeds into the vegetated drainage ditch to the right.

METHODS

Tracer Tests

Tracer tests were conducted to determine the HRT of DBR 1 and DBR 3. Firstly, expected HRT was calculated with the following formula (Kadlec and Wallace 2009):

$$\tau_{nominal} = \varepsilon(LWh)_{nominal}/Q$$

where

ε = bed media (total) porosity, dimensionless
LWh = volume, m^3
Q = flow rate, m^3/d

Bed porosity, ε, was estimated via three trials by submerging saturated woodchips taken from the bioreactors in a beaker and compressing them to simulate the weight of the water-saturated woodchips. Water was added in incremental volumes, and total water volume needed to completely submerse a premeasured volume of woodchips was recorded in each trial. The result was a porosity estimation of 0.45, and therefore an expected HRT of approximately 24 hours for all three bioreactors.

Tracer tests were conducted between June and October, 2013 to evaluate the HRT of the bioreactors and other wetland hydrological parameters (Table 2). During the tracer tests, inlet flow rate from the gauge, total liters in, and outlet flow rate via a bucket test were recorded one to two times a day during the first four days and at least once every two days after that. Sodium bromide (NaBr) was used as the tracer. The mass of tracer to be injected at each site was estimated using a modeling approach in R, with the goal of recovering an outlet bromide peak that was 10 to 50 times the background bromide concentration for each site (Kadlec and Wallace 2009).

Table 2. Summary of tracer tests.

Site	Date	Notes
DBR 3	Aug. 8-15	Started same day as 300 lbs. woodchips added
DBR 3	Sept. 19-27	
DBR 1	Oct. 15-19	Four days of bromide concentration data were collected due to the inlet pump malfunctioning

We injected the bromide salt into the bioreactors at a concentration of 6.2 g/L, which was diluted further by the normal inlet water entering the bioreactor. A total of 1.2 kg NaBr was injected at the smaller bioreactors, DBR 2 and DBR 3, and 3 kg NaBr at DBR 1. The bromide solution was injected at the inlet at the same flow rate of the bioreactor influent, during which time the normal inlet flow was switched off; tracer pouring was alternated with turning back on the normal inlet flow at 10-minute intervals throughout the tracer injection period. The total duration of tracer injection was 1 hour, 15 minutes at DBR 1, and 2 hours, 30 minutes at DBR 3 due to the difference in flow rates.

We collected outlet samples for approximately one week after adding the tracer, using an autosampler (ISCO 6700, Teledyne, Lincoln, Nebraska). The autosampler was programmed to take samples hourly for the first four days for the DBR 1 test and the second DBR 3 test; during the first DBR 3 test, which was the first tracer conducted, it was programmed to sample every 3 hours for the first 24 hours. After four days, samples were taken every two hours at both sites. The 100 mL-samples were transferred from the autosampler into high-density plastic bottles that were brought back to be stored in lab until analysis.

Internal sampling was also conducted during the first day after tracer injection, to monitor potential tracer stratification. Samples were taken from multiple depths from the three monitoring wells within the first 5.5 m along the bed, at the tracer test start time and after 24 hours. All samples were filtered with 0.45 µm filters fitted to syringes and were stored in lab for up to one month before analysis. Samples were analyzed for bromide using an ion chromatograph (ICS-2000, Dionex, Sunnyvale, CA). Quality control samples checked to be within 20% of intended concentration.

A plot of bromide concentrations was then generated for each tracer test, after normalizing the bromide concentration for each sample to the mean flow rate during the interval that the sample was taken. The mean ratio of the time of the tracer peak to the mean HRT was 4:5 for subsurface flow wetlands and was used to quantify mean HRT (Kadlec and Wallace 2009).

The reason for using peak time as a proxy instead of a model-based approach or geometric calculation method was that these other two methods were deemed

inappropriate for the tracers conducted. The number-of-tanks-in-series, or NTIS, model was considered for this study since it is one tool used to assess wetland hydrology, but other bioreactor studies did not use this approach and therefore the mathematical fitting parameters the model produced could not be used comparatively. Geometric moment analysis has been used to report bioreactor tracer test results in some studies, but this approach is considered antiquated due to overemphasis of the tracer tail (Cameron and Schipper 2012; Kadlec and Wallace 2009).

Monitoring of nitrate and dissolved organic carbon (DOC)

We collected water samples for analysis of nitrate and dissolved organic carbon once a week from DBR 2 to evaluate spatial patterns, with the potential to assess dead zones and bypass flow. Some samples were additionally analyzed for ammonium. The sampling occurred primarily during the summer and fall of 2012.

At each sampling event, 100-mL samples for nitrate and 50-mL TOC samples were collected in random order from each of the monitoring well ports in the bioreactors at the 0.3-meter depth, 0.6-meter depth, inlet and outlet. The intake piece of each port was fitted with an aquarium bubbler to exclude large particles during sampling.

Samples for nitrate were also collected during the tracer tests from the bioreactor inlet and outlet, for two days at DBR 1 and three days at DBR 3. These samples were collected by the ISCO autosampler, this time with the center console filled with ice packs. Samples were brought back to lab twice each day and ice packs replaced. Due to the short sampling duration, analysis of these results was limited and can be found in Appendix B.

Nitrate samples were filtered through a 0.45 µm Whatman filter, stored in plastic bottles and frozen until analysis on a flow injection analyzer (QuikChem 8500, Lachat Instruments, Loveland, CO). Dissolved organic carbon (DOC) samples were collected in amber glass bottles, and were acidified and refrigerated until analysis on a Shimadzu Combustion TOC Analyzer (TOC-$V_{CPH/CPN}$, Shimadzu Corporation, Kyoto, Japan).

A comparison of nitrate levels was conducted between 0.3 meter and 0.6 meter depths, using a one-sided paired t-test. These data did have a normal distribution, as shown by a Shapiro-Wilk test ($p=0.22$). Similar analysis was conducted for dissolved

organic carbon; conditions for nonparametric Mann-Whitney U test were met. Nitrate levels were also compared across the width of the bioreactor at the midway set of monitoring wells by Kruskal-Wallis rank sum tests. Samples for width comparison were made at 5.5 m along the length of the bioreactor, using three monitoring wells that were side-by-side and roughly 0.3 m apart.

Volumetric nitrate removal

A simple calculation was performed to convert UC Cooperative Extension-reported nitrate removal (mg/L/day) to volumetric removal (g/m^3 bioreactor volume/day), using the volume of water passing through an estimated half the bioreactor volume per day:

$$\frac{8\ mg\ NO_3}{day} \times \frac{7.6\ L}{min} \times \frac{60\ min}{hour} \times \frac{24\ hours}{day} \times \frac{1\ day\ res\ time}{13\ m^3\ bioreactor\ media} \times \frac{1\ g}{1000\ mg}$$

This conversion was calculated to compare the DBR 1 removal rate to other studies' reported removal rates, equalized by volume of media.

Nitrous Oxide (atmospheric flux and dissolved)

We collected samples weekly for two months, from July 10 through September 20, 2013, at DBR 2 and DBR 3. Eight nitrous oxide sampling events were completed for DBR 2 and seven sampling events for DBR 3. Fluxes from the bed surface and dissolved nitrous oxide samples were collected during the same sampling event.

Atmospheric flux

We measured atmospheric flux using three static chambers—near-inlet, mid-bed, and near-outlet (Fig. 3). Chambers were placed at least 0.3 meters away from the edge of the bioreactor to allow access without disturbing the bioreactor and potentially influencing gas emissions.

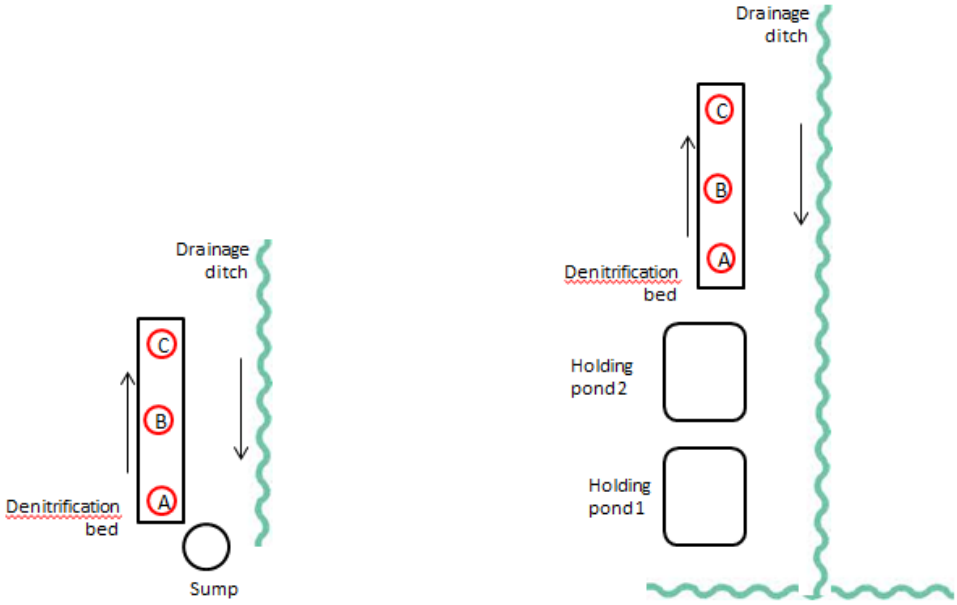


Figure 3. Placement of chambers at each field site, DBR 2 (left) and DBR 3 (right). Chamber A was located within a meter of the bioreactor inlet, chamber B about 5.5 m from the inlet, and chamber C near the outlet.

PVC bases that were 0.05 m in diameter and static, vented chambers that were roughly 8 liters were used during sampling per the methodology of Parkin and Venterea (2003) and Rochette and Eriksen-Hammel (2008). Bases were inserted to a depth of 8 cm, and were installed at least 24 hours before sampling. Chambers were wrapped in gold mylar reflective tape to minimize the effect of the sun on internal chamber temperature (Parkin and Venterea 2003).

During the sampling event, five gas samples were taken at eight-minute intervals, for a total sampling time of 32 minutes. Samples were drawn from chambers using a 20-mL syringe and injected into 12 mL pre-evacuated glass Exetainer vials (Labco Ltd., High Wycombe, UK), resulting in overpressured vials. Samples were analyzed on a gas chromatograph (Shimadzu GC 2014, Shimadzu Corporation, Pleasanton, CA) and calibrated monthly using a standard curve. Quality control samples were run every 20 samples. The data were analyzed using the HMR package in R Statistical Software (Pederson 2013; R Core Team 2013).

Kruskal-Wallis tests were conducted to compare fluxes based on chamber location at each site; Spearman's rank sum test was used for correlation analyses.

Dissolved nitrous oxide

Samples for dissolved nitrous oxide were collected at the inlet, outlet, and midpoint of the bed as well as from the adjacent drainage ditch, upstream and downstream of the bioreactor, at both sites. Two replicates were sampled per location, and the average result of these replicates was used as the final sample value. Air and water temperatures during the sampling period were also measured for correlation analysis.

Sample collection was based on vapor-liquid equilibrium principles. First, a 25-mL water sample was drawn from the bed with the 50-mL glass syringe; the remaining headspace was filled with an equal volume of argon. Immediately after, the syringe was shaken vigorously for one minute. A 20-mL gas sample was taken from the syringe via injection into an evacuated glass vial, which was stored at room temperature until analysis on a gas chromatograph (Shimadzu GC 2014, Shimadzu Corporation, Pleasanton, CA) (Kazunori et al. 2010).

Analysis was conducted by, firstly, computing the concentration of dissolved nitrous oxide per volume of water in the sample:

$$V_{N20} = C_t[V_h + (V_{water}\alpha)] \times \frac{1\ L}{1000\ mL}$$

where V_{N20}(µL) is the volume of N_2O emitted at time *t*, C_t is the N_2O gas concentration in the gas phase at time *t*, V_h (mL) is the volume of the headspace, V_{water} (mL) is the volume of water, and α (mL N_2O per mL water) is the Bunsen absorption coefficient (Carter and Gregorich 2008). At 25 ˚C and 1 atm, α is 0.632 mL gas per mL water for nitrous oxide (Tiejde 1994). Volumetric nitrous oxide resulting from this equation was then converted to a mass-based concentration; specific volume for nitrous oxide is 0.553 m^3/kg at 25 ˚C.

Export of nitrous oxide from the bioreactors was estimated using the outlet flow rates measured during tracer tests and mean dissolved nitrous oxide levels from the bed and outlet.

RESULTS AND DISCUSSION

Tracer testing and bioreactor hydrology

Mass recoveries of bromide from the tracer test at DBR 1 and for the two tests at DBR 3 were 35%, 44% and 54%, respectively. The DBR 1 mass recovery would have been greater if the inlet pump had not malfunctioned during the test; a total recovery of 44% for DBR 1 was estimated by extrapolating the tracer curve at the same slope as for the last several hours of data (Fig. 4). Mass recovery of salts is usually calculated for tracer tests, where over 80% is considered acceptable (Kadlec and Wallace 2009). We believe that when the tracer was injected, some of the bromide salt sank and moved through the bioreactor more slowly or was detained somewhere in the bed, and was not detected during the sampling interval. It may also be possible that some of the bromide was delayed due to woodchip media adsorbing the bromide, even though bromide is typically a conservative tracer that does not 'stick' to soils and plants in a wetland environment (Kadlec and Wallace 2009). The background bromide concentration at the sites ranged between 0.7 and 1.3 mg/L Br.

Two tracers were conducted after the annual woodchip replenishment, when the porosity was expected to represent the most optimal bioreactor functionality, at DBR 1 and DBR 3. For DBR 1, the peak of the tracer curve occurred at 41 hours; the calculated mean HRT for the bioreactor was 51 hours. This HRT was more than double the expected retention time of 24 hours. The HRT of the tracer conducted at DBR 3 was 35 hours (tracer peak time of 28 hours), which was 1.5 times longer than expected.

Although the DBR 1 HRT was longer than that of DBR 3, this result was confounded by differential tracer mixing between sites; there was evidence of less bromide stratification between depths at DBR 1 than at DBR 3 (see next sub-section for more details of this assessment). Therefore, the DBR 1 bromide plot provided results that were more accurate because they represented the hydrology of a greater proportion of the bed volume.

Visual assessment of both bromide plots shows that the tails do not decrease smoothly, but rather have intervals of increasing bromide concentrations after the main peak, indicating that the tracer was temporarily detained somewhere in the beds (Fig. 4).

Although this detention in some cases could be considered evidence of preferential flow paths, tracer stratification during our tests confounds this evidence, as tracer detention may not represent true bed hydrology. The bromide tracer may have been detained behind one of the baffles or pooled at the end of the bed below the outlet structure, and also could have temporarily adsorbed to the woodchips (Kadlec and Wallace 2009; Chazarenc et al. 2003).

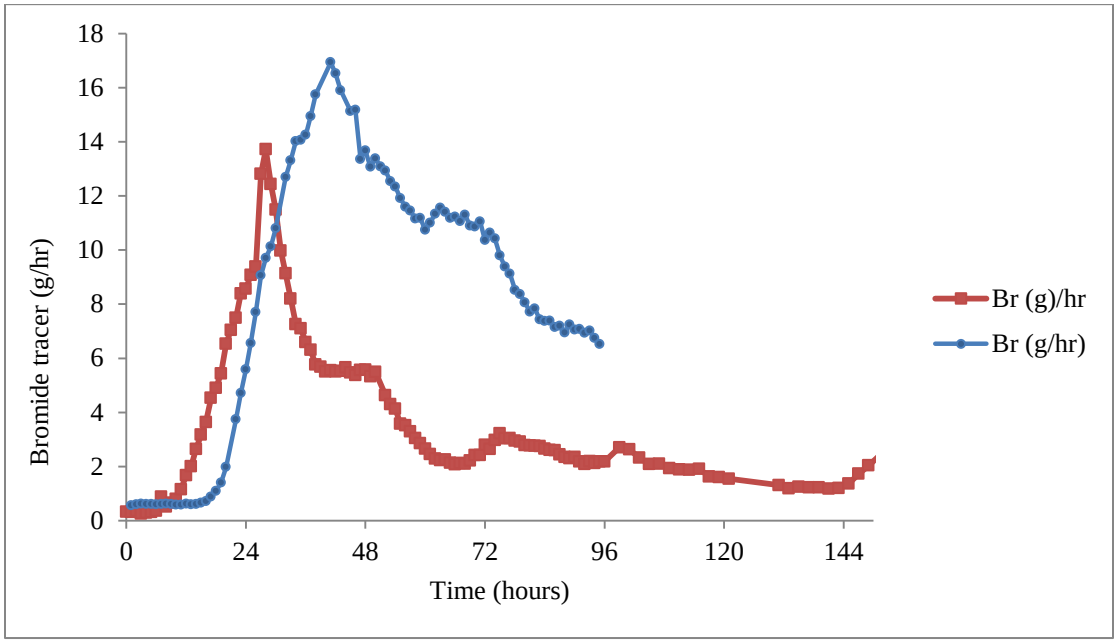


Figure 4. Bromide tracer plots based on outlet samples collected hourly.

An additional tracer test, conducted at DBR 3 before woodchip replenishment, showed that the low woodchip density and corresponding higher porosity observed in the half of the bioreactor nearest the inlet did result in a much shorter HRT than in the post-woodchip replenishment tracer at DBR 3-- tracer peak times of 13± 2 hours versus 28 hours, respectively (Appendix A).

Implications of the longer-than-expected DBR 1 HRT suggest that the porosity was greater than in preliminary calculations, but unfortunately the HRT also suggests a porosity that is greater than is feasible. The HRT-based calculation of porosity is 0.89, which is nearly all water, even though the bed was packed very tightly with woodchips. Porosity for woodchip bioreactors from other studies were estimated at 0.65 for softwood and 0.7 for hardwood, based on tracer tests (Robertson 2000; van Driel et al. 2006). Using tracer peak time from the bromide plot for this bioreactor would suggest a porosity of 0.72, which is more reasonable.

Therefore, estimating porosity to within a reasonable range of values was used as a tool to confirm the HRT estimation method. In our case, determining HRT via a published ratio for subsurface wetlands was shown to be unreliable as it overestimated both HRT and porosity in the bioreactor. Using tracer peak time as a proxy for HRT resulted in more ostensibly accurate HRT and porosity values. Proposed explanations for the incongruity between estimating HRT for subsurface wetlands and woodchip bioreactors using the tracer-peak-time-to-HRT ratio from subsurface wetland studies include: differences in tracer behavior between true subsurface wetlands and woodchip bioreactors, or differences between the systems themselves. If the latter, wetland size and also the presence of soil and plants are proposed key differences. Tracer stratification, with higher concentrations of bromide at the lower depths of the bed, could also have affected HRT, since not all of the bed depth intervals were equally represented.

Tracer stratification and future recommendations

Although outlet bromide concentrations were within the range recommended by Kadlec and Wallace (2009), some unfavorable tracer stratification occurred during the tracer tests. There appeared to be a lesser degree of tracer stratification at DBR 1 compared to DBR 3, based on a limited number of internal monitoring samples (Table 3).

We postulate that there was a higher degree of tracer stratification at DBR 3 due to the slower inlet flow rate, as well as downward-forcing baffle placement and possibly differences in the dimensions of the bioreactors. A study that conducted a tracer test in a

Table 3. Internal monitoring of tracer concentrations. All bromide samples presented here were taken at a monitoring well located 5.5 m from the inlet at each site.

			Bromide (mg/L)		
Site	Time since tracer injection (hours)	Baffle placement relative to monitoring well	0.3-m depth	0.6-m depth	0.9-m depth*
DBR 1	22	0.3 m after upward-forcing baffle	35.8	40.3	50.9
DBR 3- before woodchips added	25	1.2 m after upward-forcing baffle	10.1	10.6	33.9
	30		12.5	14.7	26.9
DBR 3- after woodchips added	24	1.2 m after upward-forcing baffle	9.5	3.2	40.2

***The deepest sampling point of 0.9 m was slightly shallower than actual bed depths of 1.2 m for DBR 1 and 1.1 m for DBR 3; due to the tracer gradient observed, concentrations would be highest at the bottom of the beds.**

subsurface wetland reported tracer sinking at flows less than 2 m^3 h^{-1}, equivalent to 33 liters/min (Chazarenc et al. 2003). While differences between a subsurface wetland and woodchip bioreactor due to such factors as porosity and media properties are likely to affect tracer density in different ways, there is likely also some minimum flow rate below which salt tracers should not be conducted in woodchip media due to sinking. Downward forcing baffles at DBR 2 and DBR 3 immediately after the inlet may also have contributed to differential degrees of tracer sinking at these sites.

However, tracer stratification at both sites may be attributed to inadequate mixing of the tracer solution in the bed water column during tracer injection, resulting in density issues with the high concentrations of tracer (Kadlec and Wallace 2009). Tracer mixing during injection is difficult in systems such as woodchip beds that have no open water in which to manually mix the entering solution. Injecting a smaller total mass of bromide may have helped to reduce inlet density issues.

Although tracer stratification occurred at both sites, particularly at DBR 3, even the highest bromide tracer concentrations observed at the mid-bed sampling location were two to three times lower than the highest nitrate concentrations at the sites. The molar mass of the two ions are similar. Therefore, the bromide concentrations at the midway length along the bioreactor are not high enough to incur tracer sinking, but as previously suggested, other factors may have caused the tracer to stratify in the near-inlet area of the bioreactor.

Further experiments on flow rate versus tracer stratification in woodchip bioreactors are recommended, as is monitoring tracer concentrations within the bed at as many locations and time intervals as possible during tracer testing. More internal monitoring would also have been useful in locating tracer that was not recovered during the sampling interval in this study. Bed design features such as baffles may also detrimentally impact tracer mixing.

Spatial patterns of nitrate and dissolved organic carbon

Nitrate concentrations in the DBR 2 bed ranged from 54 to 154 mg NO_3-N/L nitrate, with a mean of 92 mg NO_3-N/L. There was a mean difference of 4.3 mg NO_3-N/L between 0.3-m and 0.6-m depths, with greater nitrate concentrations at 0.6 m ($p=0.03$).

Nitrate concentrations did not vary perpendicular to the flow of the bioreactor, for either the 0.3-m or 0.6-m depth, at the set of wells that were located halfway along the length of the bioreactor ($p=0.83$, $p=0.30$, respectively). The uniformity of nitrate levels across the width indicates there are no major differences in nitrate removal across the width of the bioreactor, as measured at 5.5 m from the inlet.

Nitrate concentrations measured at 1.8 meter intervals along the bed did not show a constant decrease with distance from the inlet. Median concentration differences for one interval ranged from -6.6 to 13.0 mg/L N at the 0.6-meter depth, and 1.5 to 8.1 mg/L N 1.2-meter depth. The expected removal rate for a 1.8-m interval, based on average nitrate removal for the whole bed, was 2.7 mg/L. However, samples were not collected at offset time intervals needed to show removal. In addition, fluctuating inlet nitrate concentrations also confounded the ability to monitor and report nitrate removal within the bed (Appendix B). Lastly, the effect of mixing in the bed also confounded the ability to measure nitrate removal without attempting to measure or model the flow dynamics within the bed.

Dissolved organic carbon concentrations ranged from 5.1 mg/L to 10.4 mg/L at DBR 2 during the sampling period from July to November, 2012. There was on average 0.9 mg DOC/L less carbon available at the 0.6-meter depth than at 0.3 meters in DBR 2 ($p<0.001$). This trend may or may not be representative along the full bioreactor depth range of 1.4 meters.

The mean increase in dissolved organic carbon between the inlet and outlet was 2.4 mg/L. Ammonium levels were consistently less than 0.05 mg/L at all sampling locations (inlet, outlet, in-bed).

The most probable mechanism for the higher DOC concentrations at the shallower depth is the annual woodchip replenishment. The several hundred kilograms of woodchips that are added each year are added to the surface, and are thought to compress, as well as decompose, over the course of the year.

The nitrate gradient between the 0.3- and -.6 meter depths could indicate that more denitrification is occurring in the shallower depths. Two possible explanations for this pattern are the higher DOC at shallower depths, or flow patterns such that water

being treated near the surface moves more slowly than at the bottom, increasing the treatment time of the slower flow path.

Volumetric nitrate removal

Volumetric nitrate removal for DBR 1 was 6.8 g nitrate-N/m^3 woodchip media/day removal during the summer months, and is fairly high compared to other bioreactor studies. A review on woodchip bioreactors, specifically those treating subsurface agricultural drainage, reported removal rates ranging from 0.38 to 7.76 g NO_3-N/m^3 woodchip media/day, for bioreactors of similar or greater volume to this study (Christianson et al. 2012). In their review of denitrifying bioreactors, which included but were not limited woodchip media, Schipper et al. (2010) reported a range of 3.2 to 9.7 g NO_3-N/m^3 bioreactor media per day for bioreactors with a bed design that were not nitrate-limited and were larger than 2 m^3; the 9.7 g NO_3-N/m^3 bioreactor media per day was for a bioreactor that was much larger than the others at 1320 m^3.

Despite favorable comparisons to other studies, the bioreactors receiving tile drain effluent do not come close to lowering nitrate concentrations to below the MCL; an average of 12% and 19% of the influent nitrate concentrations that are frequently over 100 mg NO_3-N/L is removed by DBR 1 and DBR 2, respectively.

Other media such as maize cobs have been shown to produce higher rates of denitrification and nitrate removal. Cameron and Schipper (2011) reported 21.8 g NO_3-N/m^3/day for maize cobs. However, the longevity of woodchips is an advantage, and local availability of media is also a factor. The UC Cooperative Extension has tested methanol injection into the woodchip beds to increase nitrate removal, which may be a cost-effective alternative; their data showed more than double the nitrate removal rates at 20 mg/L methanol (Hartz 2014).

Nitrous oxide emissions from beds

Atmospheric flux

Mean flux per chamber location ranged from 250±310 µg N_2O-N/m^2/hour (mean ± 1 SD) to 2,700±1,500 µg N_2O-N/m^2/hour at DBR 2 (Figure 5*a*). DBR 3 mean fluxes ranged from 30±17 µg N_2O-N/m^2/hour to 2,300±1,500 µg N_2O-N/m^2/hour (Figure 5*b*).

The midpoint chamber at DBR 3 was excluded from statistical analysis; we posit that the base at this chamber location could not be considered 'sealed' due to a higher pile of unsaturated woodchips at this chamber location. Chambers and bases should be 'sealed' over the particular sampling location to capture accurate nitrous oxide fluxes (Rochette and Eriksen-Hamel 2008).

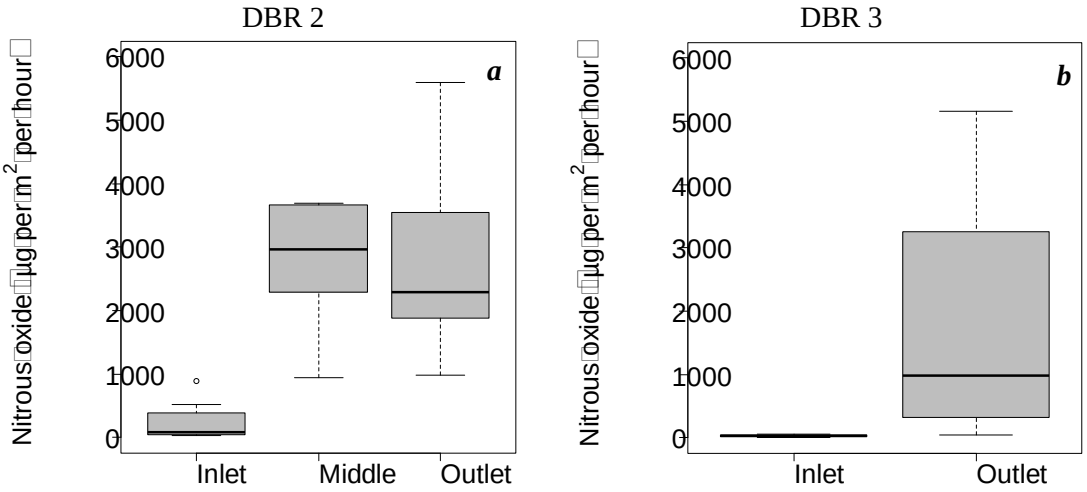


Figure 5*a* and 5*b*. Surface nitrous oxide emissions from data collected weekly from July 16 through September 20, 2013. The mid-bed chamber at DBR 3 (right) is not pictured because issues with that data, due to difficulty of 'sealing' the chamber base in several inches of dry woodchips at that particular location.

There was a significant difference among fluxes depending on chamber location at both DBR 2 and DBR 3 ($p<0.001$, $p=0.02$, respectively). The near-inlet fluxes appear to be much lower than the other chamber(s) at both sites.

No correlation between bed temperature and surface flux was present at DBR 2 ($p=0.28$). The mean bed temperature of DBR 3 was 2.2 ˚C warmer than mean DBR 2 bed temperature. pH in the denitrification beds had a small range of 6.9 to 7.3.

Two other woodchip bed studies reported very different atmospheric nitrous oxide fluxes from each other. Warneke et al. (2011a) reported an average 4,716 µg N_2O-N/m^2/hour for a 176-m long bed with effluent temperatures ranging from 15.5 to 23.7 ˚C; nitrate concentrations entering the bed ranged from 50 to 150 mg NO_3-N/L, depending on the season. They recommended exploring mechanisms to reduce the amount of nitrous oxide as well as the other greenhouse gases emitted from denitrification beds if they

became commonly used, based on these emissions, which are of similar magnitude to our study.

Conversely, the mean summertime flux from a bed in Illinois was 10 to 130 µg N_2O-N/m^2/hour (Woli et al. 2010). The influent nitrate concentrations of their bed ranged from 2.8 to 18.9 mg NO_3-N/L, which decreased to 0.1 to 14.6 mg NO_3-N/L at the outlet. They reported soil temperatures of up to 25 ˚C in the summer, but did not report water temperature in the bioreactor.

Mean fluxes from treatment wetlands receiving either agricultural wastewater or sewage are reported to be 130 to 280 µg N_2O-N/m^2/hour (Fey et al. 1999; Johansson et al. 2003). Nitrous oxide emissions may be lower with complete nitrate removal, which was not achieved by either bioreactor; a stream-bed denitrifying bioreactor study showed lower dissolved nitrous-oxide-to-nitrate ratios when nitrate was removed to a level below 5 µg NO_3-N/liter (Elgood et al. 2010). Also, a positive correlation between nitrous oxide emissions and total nitrogen has been shown, which suggest one explanation for our results (Liikanen et al. 2006).

The near-inlet nitrous oxide fluxes, which were one to two orders of magnitude less than near-outlet fluxes, could indicate that low rates of denitrification are occurring within the first meter of the beds. The presence of dissolved oxygen in the inlet water is likely to largely inhibit denitrification, which only occurs under anoxic conditions, although anoxia was confirmed three meters from the inlet via a sampling port (Hartz *pers. comm.*). Additionally, the larger mean inlet emissions at DBR 2 than DBR 3 may reflect injection of methanol at the inlet for one month during the DBR 2 nitrous oxide sampling interval.

Dissolved nitrous oxide

Mean dissolved nitrous oxide from within the bioreactor beds (mid-bed, outlet) were considerably higher than samples taken from outside of the bioreactor (inlet, nearby drainage ditch) at both DBR 2 and DBR 3 (Table 4). The inlet and drainage ditch dissolved nitrous oxide levels at DBR 2 were approximately 1 percent of the outlet levels, and 1.6 to 3.4% of mid-bed levels. The mean upstream drainage ditch measurement was equivalent to only 0.1% of the highest measurement from within the DBR 3 bed.

Table 4. Mean dissolved nitrous oxide measurements (µg N per liter water) for all the sampling dates, July through September 2013, at the inlet, in-bed, outlet, and nearby drainage ditches of each DBR 2 and DBR 3. No data is available when there were not at least three viable samples analyzed (ND = no data).

	DBR 2	DBR 3
Inlet	23.3	ND
Middle	1,260	812
Middle- 2' depth	580	1,420
Outlet	1,860	932
Drainage ditch- above	17.0	1.33
Drainage ditch- below	23.3	ND

A comparison of hourly atmospheric flux for total bed volume to dissolved nitrous oxide being exported via the outlet showed that the latter accounts for an order of magnitude more nitrous oxide emissions (Table 5; Appendix F for calculations).

Table 5. Dissolved nitrous oxide, surface flux comparison, and dissolved nitrous-oxide-to-nitrate-removed ratio. All values are reported in µg N, except for the ratio which is a percent.

	DBR 2	DBR 3
Dissolved nitrous oxide (µg)		
Per square meter[a]	1,000,000	737,000
Export from outlet, hourly[b]	424,000	200,000
Atmospheric flux per square meter, hourly (µg)		
Near-inlet	100[c]	30
Mid-bed to outlet	2,710	1,790
Whole bed flux estimate	24,600	19,500
Dissolved nitrous oxide-to-nitrate ratio (%)[d]	6.2	5.3

[a] **Assuming a porosity of 0.72 and depth of 1 m**
[b] **Flow rate for the hourly export was 3.8 liters/min.**
[c] **Excluding dates of methanol injection**
[d] **Nitrate is per concentration of nitrate removed**

Dissolved nitrous oxide in these Salinas Valley bioreactors was much higher than in woodchip bioreactors from several other studies, although design type is likely to explain some of the differences, e.g. wall versus bed. Walls have vertical, downward

flow. Mean dissolved nitrous oxide in a woodchip wall that received tile drain water ranged from 13.5 to 73.2 µg N_2O-N/L; nitrous oxide at their control site ranged from 2.64 to 45.2 µg N_2O-N/L (Moorman et al. 2010). Influent water temperature and nitrate concentrations were 10.1 ˚C and 20 to 25 mg NO_3-N/L, respectively.

Dissolved nitrous oxide in a woodchip bioreactor in Ontario, Canada, ranged from -5.9 to 22 µg N_2O-N/L (Elgood et al. 2010). The nitrate concentrations in this bioreactor were maximum 6 mg NO_3-N/L, which is much lower than our inlet concentrations of 25 to 120 mg NO_3-N/L, depending on the site.

Higher dissolved nitrous oxide concentrations were reported for the 175-m long denitrification bed in New Zealand (Warneke et al. 2011a). They reported a mean release of 362,000,000 µg dissolved N_2O-N/day and up to 510,000,000 µg N_2O-N/day during the warm season from the bed. Their effluent flow rate was approximately 20 times faster than our bioreactors, while their daily dissolved nitrous oxide export was two orders of magnitude greater than for our bioreactors. (See Appendix C for further comparison of dissolved nitrous oxide in other systems such as wetlands.)

The dissolved-nitrous-oxide-to-nitrate-removed ratios for DBR 2 and DBR 3 were 5.9 and 4.9%, respectively. The 2006 IPCC ratio of nitrous oxide emissions to nitrogen (leached/runoff) is 0.0075 on a per mass basis, or 0.75%, which is a scaled-back version of their original estimate (Kazunori et al. 2010). Three other wood-based bioreactor studies reported diverse ratios of 0.003% to 3.3% (Greenan et al. 2009; Elgood et al. 2010; Moorman et al. 2010; Warneke et al. 2011a).

Our dissolved nitrous oxide method appeared to be fairly robust in that similar values for replicates were achieved, based on a rough visual assessment. However, one potential source of error in nitrous oxide analysis was our use of argon instead of nitrogen during the procedure for equilibrating the dissolved nitrous oxide with an inert gas headspace, which differed from Kazunori et al (2010). Argon is 2.5 times as soluble as nitrogen gas, which may have affected the equilibrium concentration of nitrous oxide that we sampled.

Although this study did not consider alternative bioreactor designs, it should be noted that alternative flow designs exist that may influence nitrate removal efficiency. For example, Cameron and Shipper (2011) compared horizontal and vertical flow designs

and concluded that vertical, downward flow was the most effective bioreactor flow regime for bioreactors filled with maize cobs, as evidenced by nitrate removal, hydraulic efficiency, and effectiveness of solar heating. However, it should be considered that their beds were only 2.9 m^3. In another study comparing horizontal and vertical, upward-flow systems in 17-m^3 woodchip systems, nitrate removal rates were reported to be similar between design types (van Driel 2006).

Implications of adding methanol on nitrous oxide production

For the injection rate of 20 mg/L methanol used during the experiment at DBR 2, there was no difference in levels of atmospheric flux between when methanol was being injected and when it was not (p=0.64). Dissolved nitrous oxide levels with and without methanol injection are nearly identical (Table 6).

Table 6. Mean atmospheric flux and dissolved nitrous oxide levels at DBR 2 with and without methanol injection (20 mg/L injection rate).

	Methanol	**Non-methanol**
Atmospheric flux ($\mu g\ N_2O\text{-}N/m^2/hour$)	3,200±1,700 (*n*=4)	2,500±1,100 (*n*=10)
Dissolved nitrous oxide ($\mu g\ N_2O\text{-}N/L$)		
Mid-bed	2,000 (*n*=4)	2,000 (*n*=5)
Outlet	1,100 (*n*=2)	1,000 (*n*=4)

CONCLUSION

We used tracer testing, bed monitoring of nitrate and carbon concentrations, volumetric nitrate removal calculations, and nitrous oxide monitoring to quantitatively assess the efficiency of three woodchip bioreactors in the Salinas Valley.

Using a salt tracer test, the measured HRTs were longer than expected for both DBR 1 and DBR 3. Evidence of tracer stratification at both sites that the tracers were conducted indicated that the bioreactors were not ideal systems to conduct a salt tracer, possibly due to slow flow rates that allowed the salt to sink in the water column, and also the presence of a downward-forcing baffle in the inlet area of DBR 3 that almost immediately forced the tracer to the bottom of the bed. In the future, lower concentrations, better mixing methods or a different tracer type might generate more reliable results in woodchip media with similar flow rates. In addition, we found that monitoring the tracer within the bioreactor provided information on tracer stratification; unfortunately we did not capture the location(s) of tracer detention in the bed, which may have been possible with more monitoring.

Higher concentrations of dissolved organic carbon near the surface of the bed are likely to reflect the annual addition of fresh woodchips to the surface of the beds. The spatial patterns of carbon availability may impact denitrification rates since carbon is often limited in woodchip systems. Nitrate concentrations were higher at lower depths in the bed, suggesting that denitrification rates might be lower with depth or that flow patterns near the surface allow for a greater amount of denitrification.

In spite of DBR 1's relatively high volumetric removal rate (6.7 g NO_3-N/m^3/day) compared to reported ranges 0.38 to 7.76 g NO_3-N/m^3/day for other field woodchip bioreactors, nitrate concentrations at the outlet still greatly exceed the MCL for nitrate at the two tile drain sites. Averages of 12% and 19% of the influent nitrate concentrations starting at over 100 mg NO_3-N/L are removed by DBR 1 and DBR 2, respectively. Optimization of the woodchip reactors may be possible by injecting methanol or some other supplemental carbon source into the bioreactors or increasing the temperature of the water entering the bioreactors.

Nitrous oxide emissions from the bioreactors were relatively high, both as atmospheric fluxes (2,710±1,290 µg N_2O-N/m^2/hour for DBR 2) and as dissolved hourly

export from the bioreactor outlets (424,000 µg N_2O-N/hour and 200,000 µg N_2O-N/hour for DBR 2 and DBR 3, respectively). These findings indicate that woodchip bioreactors may displace a local pollutant for a global one. Further research is needed to determine which factors are most important in minimizing nitrous oxide emissions and finding a mitigation strategy, such as determining whether complete nitrate removal in the bioreactors could reduce nitrous oxide emissions. Despite higher nitrate removal achieved by methanol injection into the bioreactors, there was no significant increase in nitrous oxide emissions during this experiment.

Based on these assessments, woodchip bioreactors have mixed potential as a nitrate-removing best management practice for the Central Coast. A higher nitrate removal rate is needed than what the current design of the woodchip bioreactors provide, although potentially-viable options such as passive solar heating and supplementing available carbon, i.e. methonal injection, are being tested to increasing removal rates. In addition, unless the production of nitrous oxide can be addressed, their value to mitigate reactive nitrogen from the environment may be limited.

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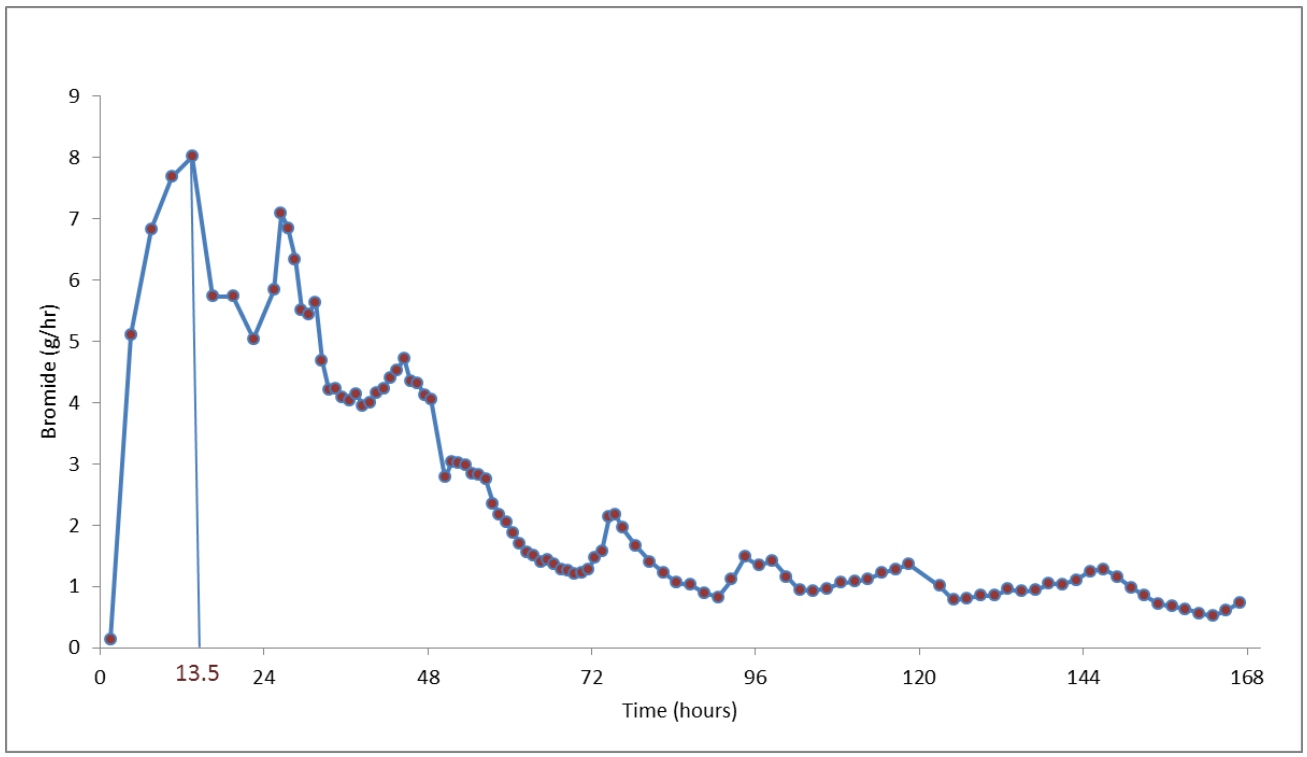
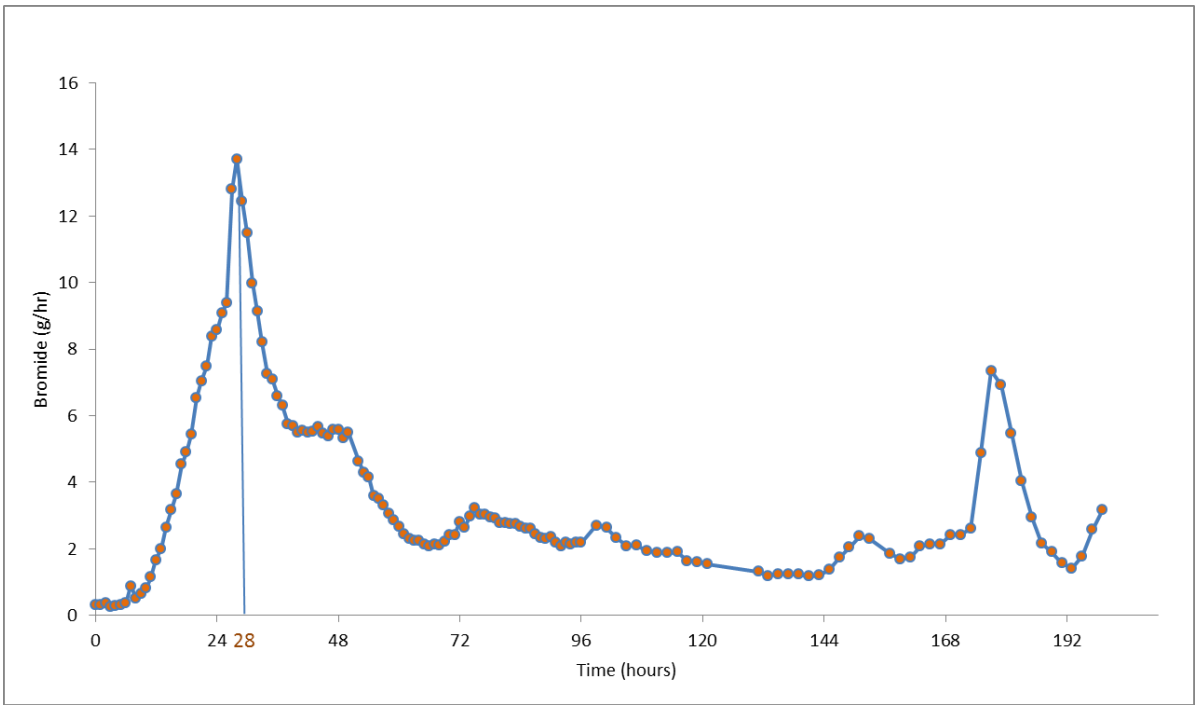
APPENDICES

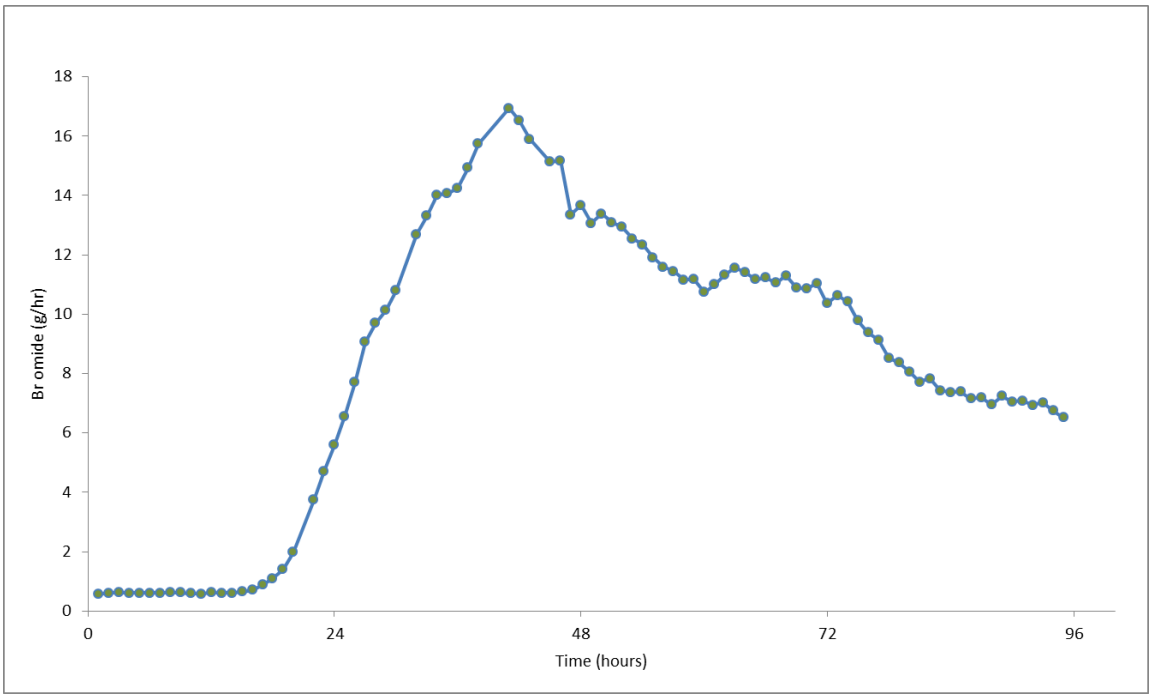
Appendix A: Tracer test bromide plots



(a) First bromide tracer at DBR 3. Tracer peak at 13.5 hours.



(b) Second bromide tracer at DBR 3.

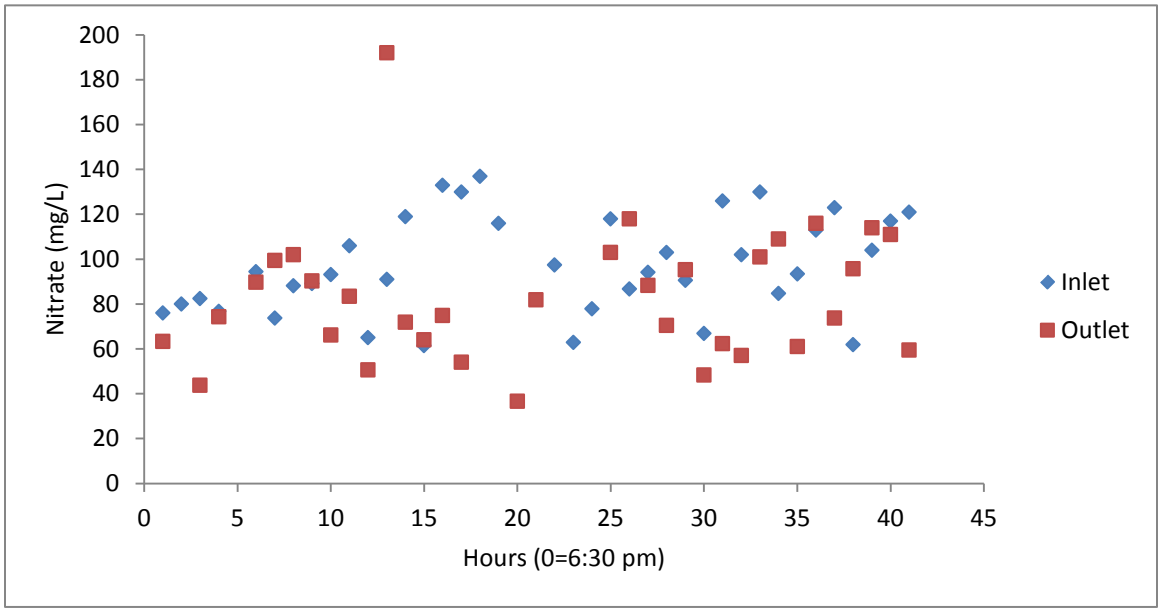


(c) Tracer test at DBR 1, incomplete due to complications with the bioreactor pump stopping four days into the test. Tracer peak at 41 hours.

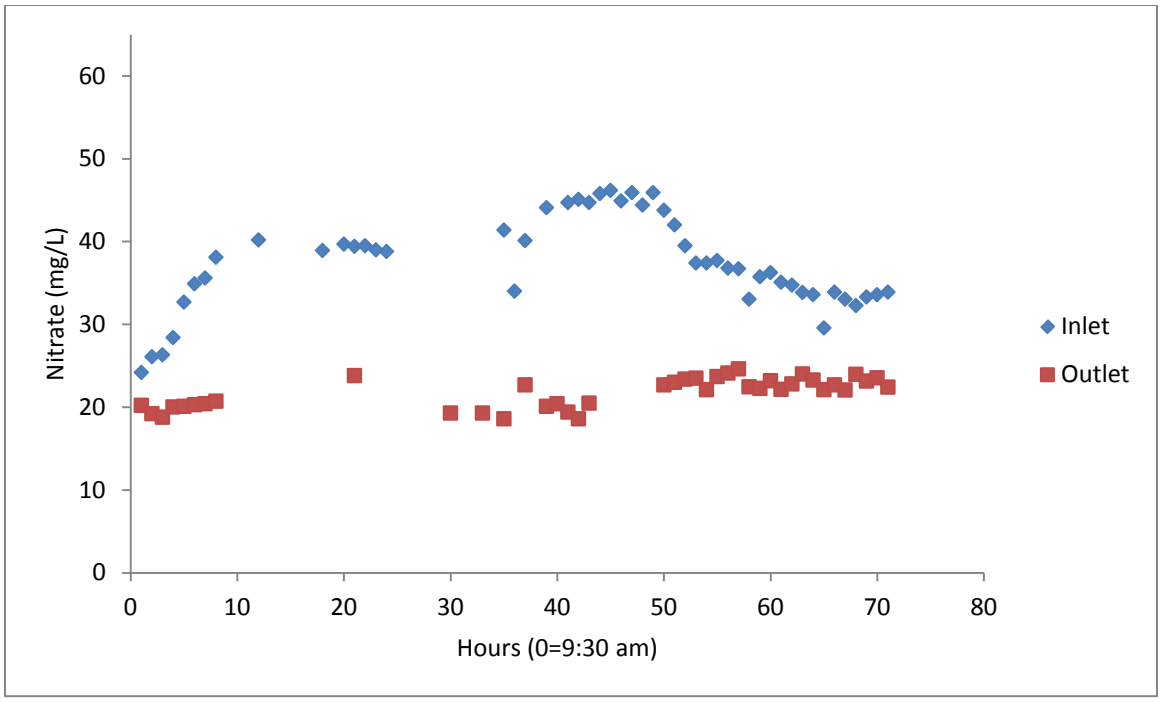
Appendix B: Hourly nitrate sampling

Inlet-outlet values are not paired in the charts below, i.e. an inlet value corresponds most closely with the outlet value either 35 or 51 hours later. No nitrate removal calculations were made based on this limited dataset.

Inlet and outlet concentrations fluctuate much more from the tile drain input source (DBR 1) than from the surface flow/holding pond input source (DBR 3). Quantitatively, the range of nitrate concentrations at the outlet is 25 times greater than the range of nitrate concentrations at DBR 3, despite the shorter data collection interval at DBR 1; the standard deviation of outlet values is 15 times greater for DBR 1 than DBR 3. Nitrate values appear to increase and decrease gradually at DBR 3.



(a) Nitrate data for DBR 1, collected 17–18 October 2013. Due to the pump shutting off unexpectedly during sampling, the sampling interval is less than even one HRT for this bioreactor. however, the data do show that both inlet and outlet nitrate concentrations change from hour-to-hour much more so than at DBR 3.



(b) Nitrate data for DBR 3, collected 3-5 October 2013. Shows consistently higher inlet concentrations than outlet, and gradual changes in nitrate concentrations due to homogeneity of nitrate levels in holding ponds feeding this bioreactor.

More collection days would be needed to determine if time of day is an important factor in inlet nitrate concentration, and additionally if a greater nitrate removal rate is observed during daytime (warmer) bed temperatures as would be expected.

Appendix C: Summary of surface nitrous oxide emission levels from several California farms and some non-local wetland studies

Category	Location	Site description and season	Emissions	Nitrate level	Comments/ Author
Farm	Salinas Co.	Lettuce at the Hartnell east campus; Chular loam; sampled several times per week when soil moisture was elevated, less frequently under dry conditions	0.58 to 1.51 kg N_2O-N ha^{-1} (6.6 to 170 µg per m^2 per hour)	Max. nitrate for 2009 was 60-100 mg N per kg soil, for 2011 was 40-60 mg N per kg soil	Interpolated daily flux measurements to get annual flux; sampling at 0, 20 40 or 0, 15, 30 if expected high; 2-year study/ Berger and Horwath, ARB report (2012)
	Yolo Co.	Cover crop, furrow irrigated plot had highest emissions; Reiff loam and Yolo silt loam; Fall/winter; reports that highest emissions occurred at beginning of rainy season (November)	Maximum mean flux was 180 µg/m^2/h for the cover crop treatment in winter; winter fluxes ranged from 50 to 180 µg/m^2/h while summer was from 20 to 80 µg/m^2/h		Cover crops conserve carbon but release nitrous oxide/ Kallenbach et al. (2010)
	Yolo Co.	One field standard tillage and one field recently converted (5 years)	0 to 23.7 g N ha^{-1} day^{-1} (99 µg per m^2		Lee et al. (2008)

		to min. tillage; Multiple seasons	per hour)		
Woodchip denitrifica -tion bed	East-central Illinois	Lined subsurface bed; April to June; soil temperature in April reported as 25 ˚C at 5 cm from the surface	10 to 130 µg per m^2 per hour		Same chamber set up (inlet, middle, outlet) as this study but chambers were smaller and possibly less accurate/ Woli et al. (2010)
Wetland	Columbus, Ohio	Surface flow riverine wetlands; multiple sub-sites were tested including high marsh, edge marsh, low marsh, and variable flow pulses; Year-round; highest fluxes recorded during the summer when soil temp. was ≥ 20 ˚C	7.0 ± 4.8 µg-N m^{-2} h^{-1} for low marsh plots; 12.6 ± 2.5 µg-N m^{-2} h^{-1} for edge plots	-	- Nitrous oxide emissions were lowest in permanently flooded plots without vegetation - Emissions increased in edge zones during and after flooding/ Hernandez and Mitsch (2006)
	Norway	Summer; subsurface flow	890 to 6,900 µg N per m^2 per day (37 to 287 µg N per m^2 per hour)		- *Greenhouse gas emissions from treatment wetlands* summary table, p. 145/ Kadlec and Wallace (2009) book, Treatment Wetlands 2nd ed.

Appendix D: Summary of several woodchip bioreactor dissolved nitrous oxide studies, two on bioreactors constructed in the field and two laboratory column studies.

Location	Study site	Dissolved nitrous oxide	Nitrate	Comments/Author
Boone Co., Iowa	Woodchip denitrification wall	Tile drain water from the control ranged from 2.64 to 45.2 µg N/L; tile drain water from the woodchip wall was 13.5 to 73.2 µg N/L; no statistically-significant difference between the two	20 – 25 ppm average in influent	Moorman et al. (2010)
Southern Ontario, Canada	Stream-bed pinechip bioreactor	Bioreactor effluent concentration range over period of study: <1 to 36 µg NL−1 Mean monthly dissolved N_2O production (difference of influent and effluent concentrations): −5.9 to 22 µg N per L	6 ppm max over 1 year span	Summer effluent temperature range: 16.7 to 19.2 ˚C/ Elgood et al. (2010)
Laboratory column study	In lab with variable water flow rates of 2.9 to 13.6 cm per day in respective	0.003 to 0.028% production of total N denitrified		Complete denitrification is stated to be occurring/ Greenan et al. (2009)

	columns			
Laboratory column study	Columns with hardwood chips, softwood chips, sawdust, greenwaste, and wheat straw	200 to 300 µg per L for the warm incubation (27 ˚C) of hardwood (eucalyptus) chips; between 50 and 100 µg per L for cold treatment (16.8 ˚C)	14.4 and 17.2 ppm at inlet of barrels	Warneke et al. (2011)

Appendix E: Summary of weekly temperature readings during nitrous oxide sampling events at each site (July 16 to September 20).

Site	Mean bed temp. (˚C)	Bed temp. range (˚C)	Mean air temp. (˚C)	Air temp. range (˚C)
DBR 2	18.4	17.1 to 19.4	20.6	17.1 to 26.1
DBR 3	20.6	19.7 to 21.8	22	19.5 to 25.3

Appendix F: Table 5 Calculations

Dissolved nitrous oxide (µg)

Convert from µL/L (volumetric) to µg/L (mass) by:

$$\frac{x\ \mu L\ N_2 0}{L\ water} \times \frac{1\ L\ N_2 0}{1{,}000\ \mu L\ N_2 0} \times \frac{1\ m^3}{1{,}000\ L} \times \frac{kg\ N_2 0}{0.553\ m^3 N_2 0} \times \frac{1{,}000{,}000{,}000\ \mu g}{1\ kg}$$

where 0.553 m^3/kg is the specific volume of nitrous oxide at 25 ˚C.

Simplifies to:
=(*x**1000)/0.553
Result (single sample) is in µg N_2O/L water.

Per square meter

- By site, multiply the average of the mid-bed and outlet dissolved nitrous oxide concentrations by 1,000 L (the number of liters in 1 cubic meter, assuming an approx. depth of 1 m for each bioreactor) by 0.72, the porosity.

Export from outlet, hourly

- Calculate average of all outlet samples per site
- Multiply result from step 2 by * 3.8 L/minute * 60 minutes.

Atmospheric flux per square meter, hourly

Convert from ug N_2O-N/ m^2/sec (HMR program result) to ug N_2O-N/ m^2/hour.

$$= HMR\ result \times \frac{60\ sec}{min} \times \frac{60\ min}{hour}$$

- For near-inlet and mid-bed to outlet estimates, respectively, take average of all values for the near-inlet fluxes by site or of the mid-bed and outlet fluxes collectively, also by site.
- For the whole bed flux estimate, take the mid-bed to outlet average and multiply by the surface area of the bioreactor (SA=13.6 m^2 for DBR 2; SA=12.8 m^2 for DBR 3).

Dissolved nitrous oxide-to-nitrate ratio

Use the average of the average dissolved nitrous oxide values at both mid-bed sampling points plus the outlet, and divide that by 20 mg/L nitrate removal for each bed.